

Fibronectin Full-length cDNA Constructs

Supplemental information for White, D.G., Hershey, H.P., Moss, J.J., Daniels, H., Tuan, R.S. and Bennett, V.D. (2003) Functional analysis of fibronectin isoforms of developing embryonic limb: Reduced Spreading of chondrogenic mesenchymal cells on full-length recombinant mesenchymal fibronectin compared to alternatively spliced isoforms. *Differentiation*. In press.

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A. cDNA Construction Schemes

All restriction enzymes were purchased from Promega and New England Biolabs. T₄ DNA ligase was purchased from Pharmacia Biotech, and alkaline phosphatase from Boehringer-Manheim. All enzymes were used in accordance with the manufacturer's instructions. See Tables 1 and 2 for primer sequences and oligonucleotide sequences, respectively. Polymerase chain reactions (PCR) were performed for 35 cycles (1 minute 94°C denaturation, 2 minute 50°C annealing, 3 minute 72°C polymerization, last cycle polymerization extended to 12 minutes) using a GeneAmp kit (Perkin Elmer Cetus) in the presence of 25 pmol of each primer. Restriction enzyme digestions were incubated from 1-16 h; fragments were identified and isolated by agarose gel electrophoresis. All DNA vectors were dephosphorylated with alkaline phosphatase, and all DNA inserts and vectors were ethanol precipitated. Ligations were incubated at 14°C for 16-64 h. Transformations of competent HB101 *E. coli* (Gibco BRL) were incubated for 30 min on ice, 45 sec at 42°C and 2 min on ice in the presence of 50 mM CaCl₂, then for 1 h at 37°C with 2 volumes of LB added. Minipreps of plasmid DNA were prepared using a Flexiprep Kit (Pharmacia), and correct insertion was confirmed by restriction enzyme digestion at alternate sites, followed by agarose gel electrophoresis. Plasmid DNA was prepared using a Plasmid Maxiprep Kit (Qiagen) and quantitated by absorbance at 280 nm.

a) prcB⁺A⁺ - Construction of the plasmid encoding the FN isoform containing both EIIIB and EIIIA. Plasmid pBAV(2) was subjected to PCR (primers C2 and D). The 300 base pair product was cut with *PstI-EcoRI* and ligated with *EcoRI-PstI* cut pBluescript SK (+). The resulting subclone was designated plasmid A.

A 3.8 kbp fragment was excised from pBAV (2) with *Nhe*I and *Hind*III and ligated with *Nhe*I-*Hin*DIII cleaved plasmid A. The resulting subclone was designated plasmid B.

Plasmid pchfn60 was subjected to PCR (primers A and B). The 238 base pair product was cut with *Bbs*I-*Sal*I and ligated with *Bsm*BI-*Sal*I cleaved plasmid B. The resulting subclone was designated plasmid C.

Annealed oligonucleotides 1 and 2 were ligated with *Bam*HI-*Pst*I cleaved pBluescript SK (+). The resulting subclone was designated plasmid D.

Plasmid pchfn22 was subjected to PCR (primers E1 and F1). The 270 base pair product was cut with *Msc*I-*Bam*HI and ligated with *Msc*I-*Bam*HI cut pchlimb7-9. The resulting subclone was designated plasmid E.

An 880 base pair fragment was excised from plasmid E with *Pst*I and *Hin*DIII. Plasmid pchfn22 was cut with *Eco*RI, then partially cut with *Pst*I for 2 minutes, yielding a 2.45 kbp fragment (complete cut yields 2.05 kbp). The 880 bp fragment and 2.45 kbp fragment were ligated with *Hin*DIII-*Eco*RI cleaved plasmid D. The resulting subclone was designated plasmid F.

Plasmid pBAV (2) was cleaved with *Afl*II, the 5' overhangs were removed with Klenow, and the DNA recut with *Eco*RI. The 400 base pair fragment was isolated and ligated with *Eco*RI-*Hpa*I cleaved plasmid F. The resulting subclone was designated plasmid G.

A 3.7 kbp fragment was excised from plasmid G with *Hin*DIII and *Sal*I and ligated with *Hin*DIII-

*Sal*I cleaved plasmid C. The resulting plasmid was designated prcB⁺A⁺.

b) prcB⁺A⁻ Construction of the plasmid encoding the FN isoform containing EIIIB.

A 1.4 kbp fragment was excised from pchfn22 with *Eco*RI and *Clal*, cut with *Tsp*5091, and a 620 base pair fragment isolated and ligated with *Eco*RI cleaved pBluescript SK (+). The resulting subclone that, when cut with *Eco*RI and *Xba*I, produced 0.65 kbp and 2.928 kbp fragments was designated plasmid N.

Annealed oligonucleotides 4 and 5 were ligated with *Bsm*BI-*Bam*HI cleaved plasmid N. The resulting subclone was designated plasmid P.

Plasmid pchfn22 was subjected to PCR (primers L and M). The 300 base pair product was cut with *Fsp*I and *Xba*I. Plasmid P was cleaved with *Bsp*MI, the 5' overhangs were removed with Klenow, and the DNA recut with *Xba*I, and ligated with the *Fsp*I-*Xba*I PCR product. The resulting subclone was designated plasmid Q.

Plasmid Q was cut with *Xba*I and partially cut with *Pst*I for 2 minutes (complete cut yields 300 base pair product. The 900 base pair partial cut product was ligated with *Pst*I-*Xba*I cleaved plasmid F. The resulting subclone was designated plasmid R.

A 1.45 kbp fragment was excised from plasmid R with *Hin*DIII and *Rsr*II and ligated with *Hin*DIII-*Rsr*II cut prcB⁺A⁺. The resulting plasmid was designated prcB⁺A⁺.

c) prcB⁻A⁺ - Construction of the plasmid encoding the FN isoform containing EIIIA.

Plasmid pchfn60 was subjected to PCR (primers H and I). The 185 base pair product was cut with *Asp*718 and *Eco*RI and ligated with *Asp*718-*Eco*RI cleaved pBluescript SK (+). The resulting subclone was designated plasmid H.

Plasmid pchlimb7-9 was subjected to PCR (primers J and K). The 315 base pair product was cut with *Eco*RV and *Xba*I and ligated into *Eco*47III-*Xba*I cleaved plasmid H. The resulting subclone was designated plasmid I.

A 520 base pair fragment was excised from pchlimb7-9 with *Eco*0109I and *Xba*I and ligated with *Eco*0109I-*Xba*I cleaved plasmid I. The resulting subclone was designated plasmid J.

*Msc*I-*Bam*HI cleaved plasmid J was ligated with the *Msc*I-*Bam*HI cut PCR product from the fifth step of the B⁺A⁺ cDNA construction. The resulting subclone was designated plasmid K.

Plasmid G was cut with *Not*I and partially cut with *Pst*I for 2 minutes (complete cut yields a 2.45 kb product). The 2.85 kb partial cut product was ligated with *Pst*I-*Not*I cleaved plasmid K. The resulting subclone was designated plasmid L.

A 3.5 kb fragment was excised from plasmid L with *Bsa*I and *Sal*II and ligated with *Bsm*BI-*Sal*II cleaved plasmid B. The resulting plasmid was designated prcB⁻A⁺.

d) prcB⁻A⁻ - Construction of the plasmid encoding the FN isoform containing neither EIIIB nor EIIIA. The 900 bp *Xba*I-*Pst*I partial cut product from the fourth step of the prcB+A- construction was ligated with *Pst*I-*Xba*I cleaved plasmid K. The resulting subclone was designated plasmid S.

Plasmid S was cleaved with *Xba*I, the 5' overhangs were removed with Klenow, the DNA recut with *Bsa*I, and the 2.2 kbp fragment was isolated. Plasmid B was cleaved with *Hin*DIII, the 5' overhangs were removed with Klenow, the DNA was recut with *Bsm*BI, and ligated with the 2.2 kbp *Xba*I-*Bsa*I fragment. The resulting subclone was designated plasmid T.

A 2.0 kbp fragment was excised from plasmid G with *Rsr*II and *Sal*II and ligated with *Rsr*II-*Sal*II cleaved plasmid T. The resulting plasmid was designated prcB⁻A⁻.

B.

Table 1
Primer Sequences

Primer designation	Sequence
A	AACAAACAACCGTCTCATGGGATAGAAGCACAACCTCC
B	AACAAACAACGTGACCAACAAAGCTTAGGTCACTGAG
C2	AACAAAGGATCCCTGCAGGAACCAACTCGTGCCACTAGCCTCCTGGAGA
D	AAACAAACGAATTCTGCTTCCTCCATAGCAGGTAC
E1	AGGAGAGCGTGCCCTGGTGGCCAGCAGACAACCGTGTAGATGTTCCA
F1	AACAAACGGATCCGTCTCCACGACCAGTTACAGCA
H	AACAAACAAGGTACCGGTCTCCTGGATAGAAGCACAAC
I	AACAAACAAGAACATTAGCGCTCTCCTGGTCATTTGACT
J	AACAAACAACGATATCCCCATCTCCAAAACCATCA
K	AACAAACAACCTAGAAGTAAGCAGTTATATCCGAGAAGTCA
L	AACAAACAAC TGCGCAATTCCACCTCCGACCAAC
M	AACAAACAAC TCTAGAGTTGTCTGTGGCATCTGTCA

The sequence of specific oligonucleotide primers utilized in PCR reactions are indicated. All primer sequences are oriented 5' to 3'.

C.

Table 2
Oligonucleotide Sequences

Oligonucleotide designation	Sequence
1	GATCCTTGTGACTACGTAACAATGCATGTTAACGGCTGCA
2	GCCGTTAACATGCATTGTTACGTAGTCGACAAG
4	TGGTCGAGACGGCTGTCACCACATGCAGGTG
5	GATCCACCTGCATGTGGTGACAGCCGTCTCG

Oligonucleotide pairs were annealed and ligated into intermediate plasmids in order to insert additional polylinker sites during recombinant plasmid cDNA construction. All sequences are oriented 5' to 3'.

D. Plasmid prcB⁺A⁺ Sequence. Length: 7635 bp

1 CTGCAGGAAC CAACTTCGTG GCCACTAGCC TCCTGGAGAG GGGCGACTCTC
51 CTCCCCATCCA CTCAAGATGC TCAGGGGTCC GGGACCCGGG CGGCTGCTGC
101 TGCTAGCAGT CCTGTGCCTG GGGACATCGG TGCCTGCAC CGAAACCAGG
151 AAGAGCAAGA GGCAGGCTCA GCAAATCGTG CAGCCTCCGT CCCCGGTGGC
201 TGTCAGTCAG AGCAAGCCTG GCTGTTTGA CAACGGGAAG CATTATCAGA
251 TAAATCAGCA GTGGGAACGG ACCTACCTAG GCAACGCCCT GGTTTGTACC
301 TGCTATGGAG GAAGCAGAGG TTTTAACACTGC GAGAGCAAGC CTGAACCTGA
351 AGAGACCTGT TTTGACAAAT ACACTGGAAA CACTTACAAA GTGGGTGACA
401 CTTATGAGCG CCCTAAAGAT TCCATGATCT GGGACTGTAC CTGCATTGGG
451 GCTGGCGAG GCAGGATCAG CTGTACCATT GCAAATCGCT GCCATGAAGG
501 GGGTCAGTCC TACAAGATTG GTGACAAGTG GAGGAGGCCA CATGAGACTG
551 GTGGCTATAT GTTGGAGTGT TTGTGTCTGG GGAATGGAAA AGGGGAATGG
601 ACCTGCAAGC CAATAGCTGA GAAATGTTT GATCACGCTG CTGGACTTC
651 CTACGTCGTG GGGGAGACCT GGGAAAAGCC CTACCAAGGC TGGATGATGG
701 TGGACTGTAC TTGTCTGGC GAAGGCAATG GGCCTATCAC CTGCACCTCC
751 CGGAACAGAT GCAATGATCA GGACACCAGG ACGTCCTACA GAATTGGAGA
801 CACATGGAGC AAGAAGGACA ACAGAGGGAA CCTGCTCCAG TGTGTCTGCA
851 CAGGCAACGG CAGAGGGGAG TGGAAGTGTG AGCGACATGT TCTACAGAGT
901 GCTTCAGCTG GATCTGGCTC CTTCACAGAT GTCCGAACAG CTATTTACCA
951 ACCCCAGACC CACCCCCAGC CCGCACCGTA CGGCCACTGT GTCACAGACA
1001 GCGGTGTGGT CTACTCTGTG GGAATGCAGT GGCTGAAGTC TCAAGGAGAC
1051 AAGCAGATGC TGTGCACTTG CCTGGCAAT GGCGTCAGCT GCCAGGAGAC
1101 AGCTGTGACC CAGACTTACG GTGGCAACTC AAACGGGGAG CCCTGTGTT
1151 TCCCGTTCA CTACAACGGT AGGACCTTCT ACTCCTGCAC CACCGAAGGG
1201 CGGCAAGACG GACATCTGTG GTGTAGCACA ACTTCAAATT ATGAACAAGA
1251 CCAGAAGTAT TCTTCTGCA CAGACCACGC GGTTTGGTT CAGACTCGAG

1301 GTGGGAATTC CAATGGTGCC TTGTGCCACT TCCCCTCCT GTACAGCAAC
1351 CGGAATTACA GCGACTGTAC TTCTGAGGGT AGGCAGGACA ACATGAAATG
1401 GTGCGGCACC ACCCAGAACT ACGATGCCGA TCAGAAGTTT GGATTCTGCC
1451 CAATGGCTGC CCATGAGGAG ATCTGCACGA CCAACGAAGG GGTCACTGTAT
1501 CGCATTGGGG ACCAGTGGGA TAAGCAGCAT GACCTGGGCC ACATGATGAG
1551 GTGCACGTGT GTTGGGAACG GCCGTGGACA ATGGGCCTGC ATCCCCTACT
1601 CCCAGCTCCG AGATCAGTGC ATCGTTGATG ACATTACTTA CAACGTCAAC
1651 GACACGTTCC ACAAGCGTCA CGAGGAGGGA CATATGCTGA ACTGTACCTG
1701 CTTCGGTCAG GGCCGGGGCA GATGGAAATG TGACCCCATC GACCGATGCC
1751 AAGATTCAAGA GACCCGGACA TTTTACCAAGA TTGGTGACTC CTGGGAGAAG
1801 TTTGTGCATG GTGTCAGATA CCAGTGTAC TGTTACGGCC GTGGCATTGG
1851 GGAGTGGCAC TGCCAGCCTC TGCAGACCTA CCCAGGCACA ACTGGACCTG
1901 TTCAAGTAAT TATCACGGAG ACCCCCCAGCC AGCCCAATTCCACCCCATC
1951 CAGTGGAAATG CCCCCGGAGCC TTCACACATC ACCAAGTACA TTCTCAGGTG
2001 GAGACCTAAA ACCTCTACGG GTCGCTGGAA GGAAGCTACC ATTCCAGGCC
2051 ACCTTAACTC CTATACCATC AAAGGCCTGA CCCCAGGTGT GATCTACGAG
2101 GGACAGCTCA TCAGCATCCA GCAGTACGGG CACCAAGAAG TGACTCGCTT
2151 TGACTTCACC ACCAGCGCCA GCACACCTGT GACCAGCAAC ACAGTGAUTG
2201 GAGAGACTGC GCCCTTTCT CCTGTTGTGG CCACTTCCGA ATCTGTCACT
2251 GAAATCACAG CCAGCAGCTT CGTGGTCTCC TGGGTCTCAG CTTCCGACAC
2301 GGTGTCAGGA TTCCGAGTGG AGTACGAAC GAGCGAGGAA GGAGATGAGC
2351 CTCAGTACCT TGATCTTCCA AGCACAGCCA CTTCTGTGAA CATTCTGAC
2401 CTGCTCCGG GCAGAAAGTA CATCGTCAAC GTCTATCAGA TATCTGAAGA
2451 GGGAAAGCAG AGCTTGATCC TGTCTACATC ACAGACTACA GCACCTGATG
2501 CGCCTCCAGA CCCTACTGTG GACCAGGTTG ATGACACTTC CATTGTTGTT
2551 CGATGGAGCA GACCCAGGC ACCTATCACA GGGTACAGGA TTGTCTATTG
2601 ACCTTCAGTA GAAGGCAGTA GCACAGAACT CAACCTTCCT GAAACGGCCA

2651 ACTCCGTCAC CCTCAGCGAC CTGCAGCCG GTGTTCAAGTA CAACATCACT
2701 ATCTATGCTG TGGAGGAGAA CCAGGAGAGC ACACCCGTT TCATCCAGCA
2751 GGAGACTACT GGCGTCCCAC GATCCGATGA TGTTCCCGCT CCAAAGGACC
2801 TACAGTTGT GGAAGTGACC GACGTGAAAG TCACCATCAT GTGGACACCT
2851 CCTAATAGCG CAGTGACTGG ATACCGTGTG GATGTCCTGC CTGTCAACCT
2901 GCCAGGGGAA CATGGGCAGA GGCTGCCTGT CAACAGGAAC ACCTTGCTG
2951 AAGTCACCGG ACTGTCCCCA GGGGTACGT ACCTCTCAA AGTCTTGCT
3001 GTGCATCAGG GCAGGGAAAG CAAGCCTCTG ACAGCACAAAC AGACCACCAA
3051 ACTCGATGCT CCCACTAACCC TCCAGTTGT CAATGAAACG GACAGAACAG
3101 TTCTGGTAAC TTGGACTCCA CCTCGAGCCC GGATAGCAGG CTACCGACTG
3151 ACAGTGGGCC TCACCCGAGG AGGCCAGCCC AAGCAGTACA ATGTGGGACC
3201 CATGGCTTCC AAGTATCCCC TGAGAAATCT GCAGCCTGGG TCTGAGTACA
3251 CTGTGACCTT GATGGCTGTG AAAGGCAACC AGCAGAGTCC CAAAGCCACC
3301 GGAGTCTTTA CTACCCTGCA GCCTCTGCGC TCCATTCCAC CTTATAACAC
3351 CGAGGTGACA GAGACCACAA TCGTGATCAC CTGGACCCCC GCTCCAAGGA
3401 TTGGCTTCAA GCTGGGTGTA CGACCAAGGC AGGGAGGTGA AGCACCCCCGA
3451 GAAGTGACTT CAGACTCAGG AAGCATCGTT GTGTCTGGCT TGACTCCAGG
3501 CGTGAATAC ACGTACACCA TCCAAGTCCT GAGGGACGGC CAGGAGAGAG
3551 ATGCACCAAT TGTCAACCGA GTAGTGACAC CGCTGTCTCC CCCAACCAAC
3601 TTGCACCTGG AGGCCAATCC TGACACTGGA GTGCTTACCG TCTCCTGGGA
3651 TAGAAGCACA ACTCCAGGCA TCAGTGGCTA CCGAGTGACC ACTGCTCCTA
3701 CAAATGGACA GCAGGGTTCT ACCTTGGAGG AAGTAGTAGG TGCAGATCAG
3751 ACCTCTTGCA CCTTGAAAAA CCTAAACCCCT GGTGTGGAGT ACAATGTCAG
3801 TGTTTACGCA GTCAAAGATG ACCAGGAGAG CATCCCCATC TCCAAAACCA
3851 TCACGCAAGA GGTGCCCAA CTCACTGACC TAAGCTTGT TGACATAACT
3901 GACTCAAGCA TCGGCCTGAG GTGGACCCCCG TTAAATGCCT CCACCATTAT
3951 TGGGTACCGC ATCACAGTAG TTGCGGCAGG AGAAAGTGTC CCTATTTTG

4001 AAGATTGT GGACTCCTCA GTAGGATACT ACACAGTGAC AGGCTGGAG
4051 CCGGGCATTG ATTACGACAT CAGTGTAAATC ACACTCATTAA ATGGCGGAGA
4101 GAGCGCCCT ACCACTCTGA CACAGCAAAC GGCGTGCCT CCTCCCACTG
4151 ATCTCCGCTT CACTAATGTG GGGCCTGATA CCATGAGAGT GACATGGACT
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4251 GAAGAAGGAG GAGGACGTTG CAGAGCTGAC AATTCACCC TCAGACAACG
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4351 TACAGTGTG TGAGCAACA TGAGAGTGCG CCTTTGTCTG GAATCCAGAA
4401 AACAGGGCTC GATTCCCCAA CTGGCCTTGA CTTCTCGGAT ATAAC TGCTA
4451 ACTCCTTCAC TGTCCACTGG ATTGCTCCTC GTGCCACCATTACGGCTAT
4501 AAGATTGGC ATCACCCGGA GCACGGTGTG GGCAGGCCA AGGAGGACCG
4551 TGTCCCCCCC TCACGAAACT CCATCACTCT CACCAACCTG CTCCCAGGAA
4601 CTGAGTATGT GGTCAGCATC ATCGCCGTCA ACGGCAGGGA GGAGAGCGTG
4651 CCCTGGTTG GCCAGCAGAC AACCGTGTCA GATGTTCAAAGGGACCTGGA
4701 AGTCAACCCCC ACCAGCCAA CCAGCCTCGA GATCTCTTGG GATGCTCCTG
4751 CAGTGACAGT CAGATACTAC CGGATCACTT ATGGTGAACAGTGGAAAGT
4801 AGCCCTGTGC AGGAGTTAC AGTGCCTGGC ACCATGTCTC GTGCTACCATTAC
4851 CACTGGCCTC AAACCCGGTG TGGACTACAC CATCACTGTG TATGCTGTAA
4901 CTGGTCGTGG AGACAGCCCT GCCAGCAGCA AGCCAGTTAC TGTCACCTAC
4951 AAAACAGAAA TAGACACGCC ATCCCAGATG CAAGTTACCG ATGTCCAGGA
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5251 ACATTCACTG AGGTGGATGT TGATTCCATC AAAATTGCTT GGGAAAGCCC
5301 GCAGGGCAA GTCACCAGGT ACAGGGTGAC CTACTCAAGC CCTGAGGATG

5351 GAATCCATGA GCTATTGCCG GCCCCAGGTG GCGAAGAAGA CACTGCTGAG
5401 CTGCATGGCC TCAGGCCAGG TTCTGAGTAC ACTATCAATA TCGTTGCCAT
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5501 TTCCACCTCC GACCAACCTG AAGTCACTC AGGTAACTCC TACCAGTCTT
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5601 AGTGAACCCC AAAGAGAAAA CCGGTCCGAT GAAAGAGATC AACCTTCTC
5651 CGGACAGCAC ATCTGCTGTC GTGTCGGGAT TAATGGTGGC AACCAAGTAT
5701 GAAGTGAGTG TGTATGCCCT GAAGGACTCC CTGACCAGCC GACCTGCCA
5751 GGGGGTGGTG ACCACTCTTG AAAATGTCAG TCCCCCTCGC AGGGCCCGCG
5801 TGACAGATGC CACAGAGACA ACCATCACCA TTACCTGGAG AACCAAGACT
5851 GAGACAATCA CTGGCTTCCA AATTGATGCC ATCCCAGCAG CAAGTGGCCA
5901 GAATCCCATC CAGAGGACCA TCAGCCCCGA TGTTAGGACT TACACTATCA
5951 CTGGCTTGCA ACCTGGCAAT GACTACAAGA TCTACCTTA CACCCTGAAT
6001 GAGAATGCC GCAGCTCTCC GGTAGTGATT GATGCCTCCA CAGCCATCGA
6051 TGCTCCTTCC AACCTGCGCT TCCTTACAAC CACAACCAAC TCCCTCCTGG
6101 CTAGCTGGCA GCCTCCTCGA GCCAAGATCA CGGGCTACAT CATTAGATAT
6151 GACAAACCTG GCTCACCAAGC CAAGGAGCTT CTGCCTCGCC CTCGGCCCGG
6201 CACCACTGAG GCTACTATTA CTGGCTTAGA ACCAGGGACC GAATACACAA
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6301 CGAAAAAGGA CAGATGATCT GCCCACGTT ATTACTGGAC CGCACCCCAA
6351 CCAGCCCGAC ATGCTCGACG TACCTTCTGT TGATGAGGGA ACCCCTTACC
6401 TCACAAACAA TAGGTATGAC AATGGAAACG GTATCCAAT TCCAGGCAC
6451 TCAGGGCACC CACAGACAAT AGGACACCAA GGTCAGCAAG TCTTCTCGA
6501 GGAGCATGGC TACAGGAGGC CTGTACCCAC GACAGCAACT CCCCTTAGGC
6551 CTGGGTCGAG ACGGCAGCCA CCAAATGTAG ATGAAGCAAT TGAAATCCCA
6601 GGGTACCAAG TGCCAATCAT AGTTGTACCT TCATACCCCC ACTCCCGTGA
6651 GCCCAGACGC AACGACACCA CGGGCCAAGA AGCTTTCT CAGACAACCA

6701 TCTCCTGGAG GCCTTGCTG GAGAGCACTG AGTACATCAT CTCATGCCAA
6751 CCAGTCAGCC AGGATGAAGA TACTTGCAG TTCAGGGTTC CTGGCACTTC
6801 CTCCAGTGCT ACGCTCACTG GCCTCACCAAG AGGTGCCACC TACAACATCA
6851 TAGTAGAACGC CCTGAAGGAT CACCGCAGAC AAAAGGTGCT GGAGGAGGTG
6901 GTCACAGTTG GCAATACTGT GTCTGAAGGA CTAACCAGC CGGCCGACGA
6951 CACCTGCTAT GATACTTACA CTGGCTCCTT CTATTCCATT GGCGAGGAGT
7001 GGGAGAGATT ATCTGAAACG GGCTTAAAC TCTGGTGCCA GTGCTTAGGC
7051 TTTGGCAGTG GTCACTTCAG ATGCGATTCC TCTAAGTGGT GCCACGATAA
7101 TGGTGTGAAC TACAAGATTG GGGAGAAGTG GGACCGGCAG GGGGAGAATG
7151 GCCAGATGAT AGACTGCACC TGCCCTGGGA ATGGAAAAGG AGAATTCAA
7201 TCGGATCCCC ATGAAGAAC GTGTTATGAC GACGGGAAGA CCTACCACGT
7251 AGGAGAACAG TGGCAGAAAG AGTATCTCGG AGCCATTGC TCCTGCACGT
7301 GTTCCGGGGG CCAGCGGGC TGGCGCTGTG ACAACTGCCG CAGACCTGGG
7351 GCTGCTGAAC CCAGTCCCGA TGGTACCACT GGCCACACCT ACAACCAGTA
7401 TACACAGAGA TACCATCAGA GAACGAACAC TAATGTAAAT TGCCCAATTG
7451 AATGCTTCAT GCCGTTGGAC GTGCAGGCTG ACAGAGATGA TTCCAGAGAG
7501 TAATCTTCAT ATCCAGCCA AGCCAACAAG TGTCTCTCTA CCAAGGTCAA
7551 TCCACACCCC AGTGATGTTA GCAGACCCTC CATTCTGAG TGGTCATTTC
7601 ACCCTAAAAA CATGCATTGT TACGTAGTCG ACAAG

E. Plasmid prcB⁺A⁻ Sequence. Length: 7365 bp

1 CTGCAGGAAC CAACTTCGTG GCCACTAGCC TCCTGGAGAG GGCGACTCTC
51 CTCCCCATCCA CTCAAGATGC TCAGGGGTCC GGGACCCGGG CGGCTGCTGC
101 TGCTAGCAGT CCTGTGCCTG GGGACATCGG TGCCTGCAC CGAAACCGGG
151 AAGAGCAAGA GGCAGGCTCA GCAAATCGTG CAGCCTCCGT CCCCGGTGGC
201 TGTCAGTCAG AGCAAGCCTG GCTGTTTGA CAACGGGAAG CATTATCAGA
251 TAAATCAGCA GTGGGAACGG ACCTACCTAG GCAACGCCCT GGTTTGTACC
301 TGCTATGGAG GAAGCAGAGG TTTTAACACTGC GAGAGCAAGC CTGAACCTGA
351 AGAGACCTGT TTTGACAAAT ACACTGGAAA CACTTACAAA GTGGGTGACA
401 CTTATGAGCG CCCTAAAGAT TCCATGATCT GGGACTGTAC CTGCATTGGG
451 GCTGGCGAG GCAGGATCAG CTGTACCATT GCAAATCGCT GCCATGAAGG
501 GGGTCAGTCC TACAAGATTG GTGACAAGTG GAGGAGGCCA CATGAGACTG
551 GTGGCTATAT GTTGGAGTGT TTGTGTCTGG GGAATGGAAA AGGGGAATGG
601 ACCTGCAAGC CAATAGCTGA GAAATGTTT GATCACGCTG CTGGACTTC
651 CTACGTCGTG GGGGAGACCT GGGAAAAGCC CTACCAAGGC TGGATGATGG
701 TGGACTGTAC TTGTCTGGC GAAGGCAATG GGCCTATCAC CTGCACCTCC
751 CGGAACAGAT GCAATGATCA GGACACCAGG ACGTCCTACA GAATTGGAGA
801 CACATGGAGC AAGAAGGACA ACAGAGGGAA CCTGCTCCAG TGTGTCTGCA
851 CAGGCAACGG CAGAGGGGAG TGGAAGTGTG AGCGACATGT TCTACAGAGT
901 GCTTCAGCTG GATCTGGCTC CTTCACAGAT GTCCGAACAG CTATTTACCA
951 ACCCCAGACC CACCCCCAGC CCGCACCGTA CGGCCACTGT GTCACAGACA
1001 GCGGTGTGGT CTACTCTGTG GGAATGCAGT GGCTGAAGTC TCAAGGAGAC
1051 AAGCAGATGC TGTGCACTTG CCTGGCAAT GGCGTCAGCT GCCAGGAGAC
1101 AGCTGTGACC CAGACTTACG GTGGCAACTC AAACGGGGAG CCCTGTGTT
1151 TCCCGTTCA CTACAACGGT AGGACCTTCT ACTCCTGCAC CACCGAAGGG
1201 CGGCAAGACG GACATCTGTG GTGTAGCACA ACTTCAAATT ATGAACAAGA
1251 CCAGAAGTAT TCTTCTGCA CAGACCACGC GGTTTGGTT CAGACTCGAG

1301 GTGGGAATTC CAATGGTGCC TTGTGCCACT TCCCCTCCT GTACAGCAAC
1351 CGGAATTACA GCGACTGTAC TTCTGAGGGT AGGCAGGACA ACATGAAATG
1401 GTGCGGCACC ACCCAGAACT ACGATGCCGA TCAGAAGTTT GGATTCTGCC
1451 CAATGGCTGC CCATGAGGAG ATCTGCACGA CCAACGAAGG GGTCACTGTAT
1501 CGCATTGGGG ACCAGTGGGA TAAGCAGCAT GACCTGGGCC ACATGATGAG
1551 GTGCACGTGT GTTGGGAACG GCCGTGGACA ATGGGCCTGC ATCCCCTACT
1601 CCCAGCTCCG AGATCAGTGC ATCGTTGATG ACATTACTTA CAACGTCAAC
1651 GACACGTTCC ACAAGCGTCA CGAGGAGGGA CATATGCTGA ACTGTACCTG
1701 CTTCGGTCAG GGCCGGGGCA GATGGAAATG TGACCCCATC GACCGATGCC
1751 AAGATTCAAGA GACCCGGACA TTTTACCAAGA TTGGTGACTC CTGGGAGAAG
1801 TTTGTGCATG GTGTCAGATA CCAGTGTAC TGTTACGGCC GTGGCATTGG
1851 GGAGTGGCAC TGCCAGCCTC TGCAGACCTA CCCAGGCACA ACTGGACCTG
1901 TTCAAGTAAT TATCACGGAG ACCCCCCAGCC AGCCCAATTCCACCCCATC
1951 CAGTGGAAATG CCCCCGGAGCC TTCACACATC ACCAAGTACA TTCTCAGGTG
2001 GAGACCTAAA ACCTCTACGG GTCGCTGGAA GGAAGCTACC ATTCCAGGCC
2051 ACCTTAACTC CTATACCATC AAAGGCCTGA CCCCAGGTGT GATCTACGAG
2101 GGACAGCTCA TCAGCATCCA GCAGTACGGG CACCAAGAAG TGACTCGCTT
2151 TGACTTCACC ACCAGCGCCA GCACACCTGT GACCAGCAAC ACAGTGAUTG
2201 GAGAGACTGC GCCCTTTCT CCTGTTGTGG CCACTTCCGA ATCTGTCACT
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2301 GGTGTCAGGA TTCCGAGTGG AGTACGAAC GAGCGAGGAA GGAGATGAGC
2351 CTCAGTACCT TGATCTTCCA AGCACAGCCA CTTCTGTGAA CATTCTGAC
2401 CTGCTCCGG GCAGAAAGTA CATCGTCAAC GTCTATCAGA TATCTGAAGA
2451 GGGAAAGCAG AGCTTGATCC TGTCTACATC ACAGACTACA GCACCTGATG
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2951 AAGTCACCGG ACTGTCCCCA GGGGTACGT ACCTCTCAA AGTCTTGCT
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3451 GAAGTGACTT CAGACTCAGG AAGCATCGTT GTGTCTGGCT TGACTCCAGG
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4251 GAAGAAGGAG GAGGACGTTG CAGAGCTGAC AATTCACCC TCAGACAACG
4301 TGGTGGTCTT AACAAATCTG CTCCCTGGTA CTGAATATCT GGTGAGAGTC
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6701 CTGGCTCCTT CTATTCCATT GGCGAGGAGT GGGAGAGATT ATCTGAAACG
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6801 ATGCGATTCC TCTAAGTGGT GCCACGATAA TGGTGTGAAC TACAAGATTG
6851 GGGAGAAGTG GGACCGGCAG GGGGAGAATG GCCAGATGAT AGACTGCACC
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7101 TGGTACCACT GGCCACACCT ACAACCAGTA TACACAGAGA TACCATCAGA
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7201 GTGCAGGCTG ACAGAGATGA TTCCAGAGAG TAATCTTCC ATCCAGCCC
7251 AGCCAACAAG TGTCTCTCTA CCAAGGTCAA TCCACACCCC AGTGATGTTA
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7351 TACGTAGTCG ACAAG

F. Plasmid prcB⁻A⁺ Sequence. Length: 7335 bp

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51 CTCCCCATCCA CTCAAGATGC TCAGGGGTCC GGGACCCGGG CGGCTGCTGC
101 TGCTAGCAGT CCTGTGCCTG GGGACATCGG TGCGCTGCAC CGAAACCGGG
151 AAGAGCAAGA GGCAGGCTCA GCAAATCGTG CAGCCTCCGT CCCCGGTGGC
201 TGTCAGTCAG AGCAAGCCTG GCTGTTTGA CAACGGGAAG CATTATCAGA
251 TAAATCAGCA GTGGGAACGG ACCTACCTAG GCAACGCCCT GGTTTGTACC
301 TGCTATGGAG GAAGCAGAGG TTTTAACACTGC GAGAGCAAGC CTGAACCTGA
351 AGAGACCTGT TTTGACAAAT ACACTGGAAA CACTTACAAA GTGGGTGACA
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6751 TTTAAACTCT GGTGCCAGTG CTTAGGCTT GGCAGTGGTC ACTTCAGATG
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7101 TACCACTGGC CACACCTACA ACCAGTATAC ACAGAGATAC CATCAGAGAA
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G. Plasmid prcB⁻A⁻ Sequence. Length: 7065 bp

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51 CTCCCCATCCA CTCAAGATGC TCAGGGGTCC GGGACCCGGG CGGCTGCTGC
101 TGCTAGCAGT CCTGTGCCTG GGGACATCGG TGCCTGCAC CGAAACCGGG
151 AAGAGCAAGA GGCAGGCTCA GCAAATCGTG CAGCCTCCGT CCCCGGTGGC
201 TGTCAGTCAG AGCAAGCCTG GCTGTTTGA CAACGGGAAG CATTATCAGA
251 TAAATCAGCA GTGGGAACGG ACCTACCTAG GCAACGCCCT GGTTTGTACC
301 TGCTATGGAG GAAGCAGAGG TTTTAACACTGC GAGAGCAAGC CTGAACCTGA
351 AGAGACCTGT TTTGACAAAT ACACTGGAAA CACTTACAAA GTGGGTGACA
401 CTTATGAGCG CCCTAAAGAT TCCATGATCT GGGACTGTAC CTGCATTGGG
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651 CTACGTCGTG GGGGAGACCT GGGAAAAGCC CTACCAAGGC TGGATGATGG
701 TGGACTGTAC TTGTCTGGC GAAGGCAATG GGCCTATCAC CTGCACCTCC
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901 GCTTCAGCTG GATCTGGCTC CTTCACAGAT GTCCGAACAG CTATTTACCA
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2401 CTGCTCCGG GCAGAAAGTA CATCGTCAAC GTCTATCAGA TATCTGAAGA
2451 GGGAAAGCAG AGCTTGATCC TGTCTACATC ACAGACTACA GCACCTGATG
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2751 GGAGACTACT GGCGTCCCAC GATCCGATGA TGTTCCCGCT CCAAAGGACC
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